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Editorial Article

Improving the chance of successful implantation – part 2 – Circumventing immune rejection and the fetal semi-allograft

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Summary

Purpose: To review possible mechanisms of how the fetal semi-allograft avoids immune rejection. Based on these mechanisms potential therapies to improve implantation by suppressing immune rejection are discussed. *Materials and Methods:* Studies supporting the importance of attaining T suppressor (sup) cells to the maternal fetal interface, while decreasing TH 17 cells along with causing a shift from cellular immunity to humoral immunity by causing a shift of influence from TH1 to TH2 cytokines is presented. Also discussed is the importance of suppressing the ability of natural killer cells to attack the fetal semi-allograft related to the secretion of an immuno-immunomodulatory protein known as the progesterone induced blocking factor (PIBF). *Results:* Progesterone supplementation in the luteal phase, possibly by causing the production especially of intracellular PIBF, may be the most important therapy to improve embryo implantation by suppressing immune rejection. Other potential therapies include human chorionic gonadotropin (hCG) supplementation and lymphocyte immunotherapy. *Conclusions:* Knowledge of the mechanism by which the fetal semi-allograft escapes immune surveillance should lead to more novel therapies to improve embryo implantation.

Key words: Progesterone; Immune surveillance; T suppressor cells; TH17 cells; Natural killer cells; Progesterone induced blocking factor.

Introduction

In part 1 of this series the authors discussed the importance of a pro-inflammatory endometrium important for trophoblast attachment to the endometrium and for adequate trophoblast invasion [1]. However, this cellular immune environment has to quickly shift to a "friendlier" environment or the fetal semi-allograft will be rejected. This editorial will discuss probable mechanisms of establishing fetal tolerance.

CD4+ CD25+ regulatory T cells

An important manuscript was published about ten years ago showing that depletion of T reg cells invariably leads to pregnancy loss unless the pregnancy was from syngeneic matings [2]. This showed that early fetal immune tolerance is dependent on T reg cells [2].

The T reg cells population starts expanding after ovulation even before implantation. Thus, this could be one of the functions of progesterone (P) since the increase occurs during the time of rising serum P levels by the corpus luteum [2, 3]. Though there is an increase in percentage of CD4+ T cells that are T reg cells from the normal 5-10% in the non-pregnant state to as high as 30% in the second trimester, there is also an increase in the percentage of decidual CD4+ T cells (about 15%) [3]. It has been demonstrated by Sasaki *et al.* that there is a lower percentage of CD4+ CD25+ regulatory T cells in early pregnancy in women who miscarry *vs.* those with normal pregnancies [4].

Interestingly, after fertilization, and possibly involving progesterone secretion, there is an accumulation of the most immunosuppressive subset of T reg cells (CCR5+ subset). Though the exact way in which these activated T reg cells suppress immune function is unknown; overall their function seems to be to negate the function of T-helper cells and dendritic cells on cellular immune function [5, 6]. Though these changes occur during the time of hormonal secretion of progesterone and estrogen in murine expression of the T reg cell population does not appear to occur to any great degree [7].

When one discusses T reg cells it is important to mention an important transcription factor, forkhead box P3 (FOXP3+) which is the main control gene for development of regulatory T cells [8, 9]. The stimulus to turn on forkhead box P3 gene activity seems to be the fetal semi-allograft [8]. In turn the transcriptional factor for the FOXP3+ gene is FOX3 which expands the T reg population [9]. Thus

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FOX3+ reg T cells have suppressive function against various white blood cells that have immune effector activity (and thus the potential to attack the fetal semi-allograft including both CD4+ and CD8+ T cells, natural killer (NK) cells, NK T cells, B cells, and dendritic cells (DCs) [10]. Other studies support the concept that it is the fetal semi-allograft or seminal plasma or both and not progesterone that causes expansion and selective of T reg cells [11,12].

The cells activated by paternal alloantigen likely increase the attraction of T reg cells to the decidua by chemokines [13]. Chemokine receptor 5 (CCR5) seems to be associated with the T reg cells attracting the most immunosuppressive cells to the decidua [13]. Thus the recommendation of most IVF centers to encourage the couple to have intercourse prior to transfer seems reasonable to possibly help implantation. T reg cells are converted from naive CD4+ T cells via certain cytokines especially transforming growth factor (TGF) beta (β) [14].

TH1/TH2 ratios and implantation

For many years the main concept in pregnancy immunology is that the pregnant state could somehow cause a shift from a dominant type 1 CD4+ T helper cell (TH17) decidual environment to a type 2 CD4+ T helper cell (TH2) type [15]. It was thought that the cytokines interleukin 2 (IL2) and interferon gamma (IFN) help to initiate cell mediated tissue damage. In contrast TH2 cells could provide "blocking" antibodies which could be protective [15]. The role of these cells in allowing implantation of the fetal semi-allograft is much less clear today.

Dendritic cells (DCs)

Dendritic cells normally help to increase T cell expansion and polarization. Therefore, it seems to make sense that they are scarce in first trimester decidua [16]. The DCs that are present seem to minimize TH1 response shifting to increasing TH2 response [17].

The maternal endometrium expresses important cytokines that are involved in suppressing immune response against the fetal semi-allograft, e.g., transforming growth factor beta (TGFβ) [18, 19]. By reducing IL6 production, it inhibits type I immune response [19, 20].

The maternal endometrium also expresses Galectin-1 (GaL-1) which is made by the endometrial stromal cells during the menstrual cycle. GaL-1 production increases significantly during the window of implantation [6]. GaL-1 is also expressed by the uterine natural killer (UNK) cell in the early pregnant uterus [21, 22]. GaL-1 may be the factor that converts some of the small DC population to a type that inhibits TH1 and TH17 response (which will be discussed in the next section) and thus may play a role in immune tolerance to the fetal semi-allograft [21, 22].

The fetal cells themselves may aid in inducing a tolerogenic phenotype in DCs through the expression of HLA-G [23]. HLA-G may also serve to cause an increase in Fas-FasL which in turn cause apoptosis of activated CD8+ T cells [24]. A future editorial on how the knowledge of immunology of pregnancy can help the fight against cancer will discuss Fas-1 and FasL-1 (known in the cancer field as programmed cell death factor-1 (PD-1) and programmed cell death ligand-1 (PDL-1) [25].

TH 17 cells

TH 17 cells develop from naïve CD4+ T cells by TGF-β, IL-6, IL-23, and IL-1 [26]. TH 17 cells secrete IL-17 (17A and 17F) which causes inflammation by neutrophil infiltration [26]. The inflammatory effect may be through the stimulation of IL-1, IL-6, IL-8, tumor necrosis factor alpha (TNFα), matrix melalloproteinase, and granulocyte machrophage colony stimulating factor (GM-CSF) [26]. Whereas there does not appear to be a difference in circulating TH17 cells in pregnant *vs.* non-pregnant women, one study found a higher proportion of TH17 cells in the decidua of pregnant women *vs.* the peripheral circulation [27]. Another study showed the accumulation of IL-17+ T cells in the decidua in women with inevitable abortions [28]. However, the data supported the theory that these cells are not the cause of miscarriage but are involved in the inflammation of the late stages of the abortive process [28]. The possibility exists however, that TH17 cells may play a role in women with recurrent miscarriage. The aforementioned study did find an increase in circulating TH17 cells and in the decidua of women with unexplained recurrent miscarriage compared to normal pregnant women [28]. Also, they found an increase in IL17 and IL23 in the serum of women with unexplained recurrent miscarriage [29].

In the discussion of T reg cells, it was suggested that these cells are needed to be present to help suppress the natural immune response to the fetal semi-allograft [30]. Indeed, these T reg are markedly reduced in women with recurrent miscarriage compared to healthy controls [30]. An important study by Liu *et al.* found an increase in the ratio of TH17 cells/CD4+, CD25 bright, FOXP3+ regulatory T cells in women with recurrent miscarriage [31]. The studies by Liu *et al.* suggest that T reg cells inhibit IL-17 expression [31]. Thus lower levels of T reg cells in the decidua may allow TH17 cells to participate in the abortive process rather than just being a later stage participant [31].

Though there had been a general concept that TH2 cytokines produce "blocking antibodies" that protect the fetus, studies have shown that there can be a deficiency of four TH2 cytokines, e.g., IL4, IL5, IL9, and IL13 and yet a successful delivery will sill occur [32]. The present concept considers the shift from TH1 to TH2 cytokines in successful pregnancy is more about reducing TH1 response (and thus cytotoxic T cells) rather than "protective antibodies" [32]. There does, however, appear to be a role for TH2 cytokines in a successful pregnancy. They seem to be able to cause the release of human chorionic gonadotropin (hCG) by extra villous trophoblast (EVT) cells. hCG may

promote T reg migration into the fetal maternal interface [33]. Insufficient levels of hCG during the luteal phase after conception are associated with decrease uterine levels of various cytokines especially FOXP3 [33].

Interestingly, failure of the corpus luteum to secrete adequate progesterone may be related to immune rejection. Erlebacter *et al.* found that TNFα may activate NK cells to destroy the corpus luteum [34].

Progesterone secretion and TH1 and NK cell function and TH1 to TH2 shifts

One of the mechanisms by which progesterone may help to prevent immune suppression of the fetus is by stimulation of the secretion of a 34 kDa protein by gamma/delta T cells in the peripheral blood streaming and in the cytoplasm of rapidly growing cells from conversion of the parent 90 kDa nucleoprotein to a 34-36 kDa splice variant [35]. The protein causes the stabilization of perforin granules in NK cells thus abrogating their cytotoxicity [36, 37].

Progesterone induced blocking factor has been shown to activate STAT-6 by binding to a novel IL-4 receptor [38]. The binding to the novel IL-4 receptors helps to change a TH1 cytokine dominant environment with cytotoxic T cells to a TH2 dominant production of IL-3, IL-4, and IL-10 [39].

The progesterone receptor modulator mifepristone was found to suppress intracytoplasmic conversion of the 90 kDa parent nucleoprotein to the 34 kDa intracytoplasmic splice variant [40]. However, interestingly the use of mifepristone or ulipristal in dosages quite sufficient to cause miscarriage did not lower serum levels of PIBF [41]. Serum levels of PIBF seem to be directly related to serum progesterone levels without the need for an allogenic stimulus [42].

Possible clinical methods to prevent immune rejection of the fetal semi-allograft leading to implantation failure: progesterone supplementation

A recent editorial in Clinical and Experimental Obstetrics and Gynecology presented in detail studies that the autjors have published demonstrating the efficacy of P not just in preventing miscarriages but in promoting fertility by preventing rejection of the fetal semi-allograft [43-46]. The use of P as an important treatment of infertility was emphasized in the editorial on infertility for the practicing gynecologist [47]. Unfortunately, there are no endometrial molecular markers that can reliably detect luteal phase lack of P secretion [48]. Unfortunately, the serum PIBF level does not seem to the method to detect inadequate P secretion since it seems that most important in the intracytoplasmic PIBF level [42].

hCG at the time of peak follicular maturation and during luteal phase

hCGs has been used for luteal phase support [49]. The theoretical mechanism in stimulating the corpus luteum to make more P and possibly estradiol (luteal phase estrogen production future topic in this series on improving the chance of successful implantation). The reason why some believe that the use of P is superior to hCG because the corpus luteum may be damaged by T reg cells and thus not respond to hCG [33].

Though the authors recently published a case report of a successful pregnancy despite an inappropriate doubling of the serum hCG levels at two-day intervals, this is rare with most cases ending in miscarriages [50, 51]. The present authors' view has been that a slow-rising hCG is the consequence of a bad pregnancy. However, the possibility exists that in some instances the low hCG levels promotes immune rejection. This then begs the question as to whether in some instances supplemental hCG injection may help implantation by helping to suppress certain immune factors not suppressed by P therapy alone. To the present authors' knowledge, there have not been any randomized controlled studies comparing the efficacy in achieving pregnancies by P support alone *vs*. P supplementation and hCG therapy in the luteal phase.

Lymphocyte immunotherapy

A previous study by Chiu *et al.* suggested that lymphocyte immunotherapy (LIT) may increase the development of P receptors in lymphocytes including gamma/delta T cells [52]. Thus the present authors considered that LIT could improve implantation by stimulating more PIBF (P interacting with increased P receptors on gamma/delta T cells [53]. However, recent data suggests that lymphocyte immunotherapy does not raise serum PIBF now that a more accurate assay has been developed [54].

Lymphocyte immunotherapy with paternal or third party lymphocytes has been demonstrated to increase T reg cells, i.e., CD4+, CD25 bright T cells [55]. Th present authors have found that LIT given to women with an average of 4.3 previous failures to conceive despite in vitro fertilization-embryo transfer (IVF-ET), yielded a 70.3% pregnancy rate *vs*. 51.3% for the controls [56]. Thus it seemed to improve implantation. Ultimately the live delivered pregnancy rate was 45.9% *vs*. 16.2% [56]. This may play a greater role in preventing miscarriage [56]. Similarly, in donor oocyte recipients failing to conceive after two transfers (fresh or frozen) of embryos derived from donor oocytes found a 90% clinical pregnancy rate for those requiring LIT *vs*. 50% for the controls with live delivery rates of 90% *vs*. 30% [57]. Unfortunately, the largest randomized study of LIT was performed by Ober *et al.* who actually found that it seemed to increase the risk of miscarriage [58]. This has led to the banning of LIT in many countries including the United States. However, in animals refrigerating white blood cells prior to injection negated the therapeutic benefit. Ober *et al.* was the only human study using refrigerated leukocytes [58]. Refrigeration may cause a critical CD200

particle on the white blood cell to break off negating its therapeutic benefit [59].

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